EXHIBIT 9

ADVERSE EFFECTS

CNS Drugs 3 (1): 41-55, 1995
1172-7047/95/0001-0041/$07.50/0

Antiepileptic Agents and Birth Defects

Incidence, Mechanisms and Prevention

Sunao Kaneko and *Tsuyoshi Kondo*

Department of Neuropsychiatry, Hirosaki University School of Medicine, Hirosaki, Japan

Contents

Summary

An association exists between maternal use of antiepileptic drugs (AEDs) during pregnancy and birth defects in offspring. The overall malformation rate is 11.1% in offspring of AED–treated epileptic mothers, while it is 5.7% in the offspring of untreated epileptic mothers and 4.8% in those of the general population. Cardiovascular defects, facial cleftings and skeletal anomalies are the most frequently occurring AED–associated malformations. No firm patterns of specific AEDs inducing particular birth defects have been established. Nevertheless, neural tube defects may be specific malformations in infants exposed to both valproic acid (sodium valproate) and/or carbamazepine. Several minor anomalies are influenced by genetic factors.

From prospective studies a number of primary risk factors for increased incidences of congenital malformations in the offspring of epileptic mothers receiving AEDs have been identified. These include high drug dosage, high serum drug concentration, the use of AEDs with high teratogenicity potency [primidone > valproic acid > phenytoin > carbamazepine > phenobarbital (phenobarbitone)] and AED polypharmacy (especially combinations of valproic acid and

carbamazepine, and phenytoin and/or carbamazepine with or without barbiturates).

The mechanism of teratogenicity of AEDs is still being investigated, but it is postulated that epoxide intermediates and other toxic metabolites of AEDs might be involved. In addition, the folate deficiency and impaired folate metabolism caused by AEDs may contribute to the teratogenicity of these drugs.

To prevent birth defects, the use of the lowest effective AED dosage and a change from polypharmacy to monotherapy are recommended before conception. A decrease in serum AED concentrations during pregnancy does not in itself justify an increase in drug dosage. The high risk of neural tube defects in offspring exposed to valproic acid (or carbamazepine) warrants prenatal examination in pregnant women receiving this drug, such as ultrasound and amniotic fluid α-fetoprotein investigations. To reduce the risk of this malformation, replacement of conventional formulations of valproic acid with controlled release formulations and the use of folate supplementation are recommended.

The improved diagnosis and therapy of epilepsy, together with better social adjustment, means that most women with epilepsy marry and have children. While antiepileptic drug (AED) therapy should be continued throughout pregnancy (since uncontrolled seizures during pregnancy may cause serious states such as threatened abortion and fetal distress), these agents carry a risk of teratogenicity.

The possibility and occurrence of birth defects in offspring of epileptic mothers treated with AEDs are of great concern to many patients and physicians. The risks of teratogenicity have been regarded as multi-factorial. Factors such as maternal background (e.g. age at delivery, seizure type, aetiology of epilepsy, etc), genetic predispositions, seizures during pregnancy and drug treatment can affect the fetus in several ways. Among them, drug factors are most closely related to the increased incidence of malformations in offspring of epileptic mothers.

In order to prevent the occurrence of birth defects, many clinicians and researchers have intensively analysed the risk factors for teratogenicity of AEDs and have suggested several preventative measures. Increased care should be taken regarding the selection, combination and dosage regimen of AEDs in pregnant epileptic women. In this article, the birth defects associated with AEDs and their possible mechanisms are overviewed, and new findings are discussed. Furthermore, the appropriate usage of AEDs in pregnant women with epilepsy is described.

1. Transplacental Passage of Antiepileptic Drugs (AEDs)

Placental transfer of nutrients, oxygen and drugs to the fetus is clearly an important and influential factor in determining fetal organogenesis. On the basis of available data in humans, it appears that for most AEDs concentrations in the umbilical vein and in maternal venous blood are almost identical.[1] Exceptions to this are valproic acid (sodium valproate) and diazepam. Concentrations of these 2 drugs are significantly higher in umbilical cord blood than in the maternal blood – the mean concentrations of valproic acid and diazepam in cord blood are 2.4 and 1.4 times higher, respectively, than those in maternal blood at delivery.[2-4] These increased drug concentrations in cord blood were considered to be mainly due to the decreased protein binding of the drugs caused by an increase in the concentration of free fatty acids in mothers at delivery, resulting in increased placental transfer and fetal accumulation.[5] However, it is unknown whether these phenomena also occur during the early stage of pregnancy.

Based on the study of human fetus-mother pairs,[6] fetal protein binding of valproic acid in the

organogenetic period is much lower than maternal protein binding. This gradually increases over pregnancy and finally exceeds maternal protein binding at term. During the organogenetic period, therefore, exceedingly high concentrations of drugs in maternal blood may possibly lead to the situation in which unexpectedly high concentrations of free drug are transferred into and affect the fetus. Pharmacokinetically, fetal tissues act as a deep compartment during pregnancy.[7] In fact, most AEDs pass across the placenta and attain pharmacologically active concentrations in the embryo or fetus.[8]

2. Epidemiology of Birth Defects

2.1 Animal Studies

The incidence of birth defects identified from animal studies is influenced by the dosage of AEDs and species differences in susceptibility to AEDs. Therefore, these factors should always be taken into account when analysing the data from these studies. Nevertheless, animal experiments have established that most AEDs have teratogenic effects.[9,10] Massey[11] reported the increased incidence of cleft lip or palate in mice treated with phenytoin. The notorious human teratogenicity of trimethadione was also reproduced experimentally in mice.[12] Nau et al.[13] clearly showed the dose-dependent development of exencephaly in valproic acid–treated mice. The teratogenicity of phenobarbital (phenobarbitone) and diazepam has also been demonstrated in animal experiments.[14,15]

The magnitude of teratogenic potency of AEDs was compared in some experimental studies.[9,16] In terms of clinical implications, such studies may provide some data that allow recommendations for the selection of less teratogenic AEDs to be made. Chatot and coworkers[16] demonstrated that phenobarbital had the least teratogenic potency among AEDs studied, and that the teratogenic activity of valproic acid was nonresponsive to nutrient supplementation that was effective in reducing the teratogenicity of other AEDs. Sullivan and McElhatton[9] ranked the teratogenic potency of AEDs: phenytoin was the most teratogenic followed by carbamazepine and phenobarbital, while clonazepam and ethosuximide were the least teratogenic. Summarising these 2 studies, the order of teratogenic potency of AEDs is: phenytoin, valproic acid > carbamazepine > phenobarbital > clonazepam, ethosuximide. Interestingly, this order accords well with the clinical observations discussed in section 5.2.

2.2 Clinical Epidemiological Studies

There have been consistent findings of high incidences of congenital malformation in offspring of AED–treated women. Table I summarises the recent prospective studies. As can be seen, the overall malformation rate is 10.5% in offspring of epileptic mothers compared with 4.8% in the control population. Among the offspring of epileptic mothers, the malformation ratio of offspring exposed to AEDs *in utero* is higher (11.1%) than that of non-exposed offspring (5.7%).

A recent international collaborative study[29] supports the finding that the malformation rate is higher in offspring of AED–treated patients than in those of drug-free patients. These data indicate that the incidence of malformations in offspring of epileptic mothers appears to be mainly attributable to AEDs rather than to epilepsy itself.

Several drug risk factors were extracted from our early prospective study:[30]

- high drug dosage
- high serum drug concentration
- AED polypharmacy
- use of AEDs with high teratogenic potency.

Based on the knowledge of these risk factors, in our recent cohort studies,[31] the number and daily dosage of AEDs were reduced and, as much as possible, AED polypharmacy was changed to monotherapy. As a result, the incidence of malformations was significantly reduced from 13.5 to 6.2% ($p = 0.031$).[31]

We analysed possible risk factors for AED teratogenicity by comparing these 2 prospective studies.[31] In addition to drug factors such as the drug dosage (expressed as drug score) and number

Table I. Results of 12 prospective studies of the incidence of malformations in offspring of mothers with and without epilepsy (reproduced from Kaneko,[8] with permission)

Reference	Epileptic mothers (total)			Treated epileptic mothers			Untreated epileptic mothers			Control population (without epilepsy)		
	LB	MF	%	LB	MF	%	LB	MF	%	LB	MF	%
South[17]	31	2	6.5	22	2	9.1	9	0	0	7 865	190	2.4
Kuenssberg & Knox[18]	48	3	6.3	48	3	6.3				14 620	447	3.0
Hill et al.[19]	28	7	25.0	28	7	25.0				165	7	4.2
Goujard et al.[20]	42	1	2.4	39	1	2.6	3	0	0	12 691	219	1.7
Shapiro et al.[21]	305	32	10.5	102	12	11.8				49 977	3216	6.4
Granstrom & Hiilesmaa[22]	147	7	4.8	134	7	5.2	16	0	0			
Nakane[23]	179	23	12.8	164	22	13.4	15	1	6.7			
Koch et al.[24]	86	7	8.1	70	5	7.1	16	2	12.5	43	2	4.7
Bossi et al.[25]	49	4	8.2	46	4	8.7	3	0	0			
Lindhout et al.[26]	165	16	9.7	151	15	9.9	14	1	7.1			
Miyakoshi & Seino[27]	132	20	15.2	123	20	16.3	9	0	0			
Kaneko et al.[28]	192	26	13.5	172	24	14.0	20	2	10.0			
Total	**1404**	**148**	**10.5***	**1099**	**122**	**11.1***	**105**	**6**	**5.7**	**85 361**	**4081**	**4.8**

Abbreviations and symbols: LB = number of live births; MF = number of malformations; * indicates a statistically significant difference from the malformation rate in the control population ($p < 0.01$).

of AEDs administered, seizure type, maternal age at delivery and aetiology of epilepsy were also identified as risk factors. However, when data were corrected for seizure type, maternal age at delivery or aetiology of epilepsy, the difference in the incidence of malformations between the 2 studies remained significant. On the other hand, this difference disappeared when the data were corrected for the drug score or the number of AEDs. These results indicate the predominant contribution of AED factors to the occurrence of malformation in the offspring of epileptic mothers.

3. Type and Incidence of Malformation

3.1 Major Malformations

Types of abnormalities and their incidences, derived from 48 separate studies reported between 1964 and 1981, were summarised by Bossi[32] and are shown in table II. Cardiovascular defects and facial clefts, primary cleft lip with or without cleft palate, are among the malformations most frequently observed in children of epileptic women treated with AEDs. Skeletal malformations including distal limb hypoplasia, clubfoot and hip dislocation are also frequent, while CNS defects and gastrointestinal and genitourinary anomalies are less frequent.

The results of 13 retrospective studies reviewed by Janz[73] showed that in the offspring of AED–treated epileptic patients, the risk of cardiac malformations was 3 to 8 times higher than that in the control group, while children of untreated epileptic patients had no increased risk. Despite these data, so far the linkage between specific AED treatment and cardiac anomalies has not been established, and cardiac anomalies have been found in offspring exposed to various AEDs in both monotherapy and polypharmacy cases.

The incidence of cleft lip and/or palate identified in retrospective (1.6%)[32] and prospective studies (1.9%)[32] is higher than that in offspring of untreated patients and in the general population, even though orofacial clefts are quite frequent in the general population (0.2%)[32] and there may be a genetic linkage between epilepsy and these malformations.[21,74,75] These reports indicate that AEDs are a major risk factor, but that genetic influences also contribute to some extent to the occurrence of some malformations.

3.2 Minor Anomalies

Minor anomalies in AED–exposed children of epileptic mothers were first reported by Meadow.[33] Since then, many reports[34,76] have described various dysmorphic craniofacial features associated with parental AED use, including hypertelorism, inner epicanthal folds, eye slant, ptosis, strabismus, flat nasal bridge, low-set ears, and abnormalities of the extremities that include hypoplasia of the nails and distal phalanges and dermatoglyphic changes. These anomalies occur alone or in combination, and are frequently associated with prenatal and postnatal growth retardation.[77,78]

The dysmorphic features constitute the main findings of the fetal phenytoin syndrome, the barbiturate syndrome,[79,80] the trimethadione syndrome[35-37,81] and the fetal valproic acid syndrome;[82] however, they are not drug specific. On the basis of available data, the incidence of minor anomalies after prenatal AED exposure varies considerably in studies from 6 to 46%,[22,38,46,53,78] with the average of the incidences probably less than 10%.[32]

Table II. Types of congenital malformations observed in 9540 neonates of mothers with epilepsy who were treated with antiepileptic drugs [from Bossi,[32] with permission (including references [17,18,20,22-26,32-72])]

Malformation	Number	Incidence (% of live births)
Facial or ear anomalies	168	1.8
cleft lip and/or palate	155	1.6
Heart disease	169	1.8
ventricular septal defect	51	0.5
Skeletal anomalies	217	2.3
nail or digital hypoplasia	68	0.7
clubfoot	60	0.6
hip dislocation	51	0.5
CNS defects	74	0.8
microcephaly	30	0.3
hydrocephalus	11	0.1
meningomyelocele	14	0.2
Gastrointestinal malformations	86	0.9
inguinal hernia	37	0.4
Genitourinary anomalies	88	0.9

4. Drug Specificity of Malformations

4.1 Craniofacial Abnormalities

4.1.1 Trimethadione

The fetal trimethadione syndrome represents an AED–specific abnormality. German and coworkers[35] reported a family in which 4 malformed children were born to a epileptic mother who was treated with trimethadione. The mother subsequently had 2 normal children following discontinuation of the drug. Zachai et al.[36] described the characteristic craniofacial abnormalities associated with this syndrome – microcephaly, v-shaped eyebrows, epicanthal folds, low-set ears and irregular teeth. Developmental delay and congenital heart defects were also confirmed by Feldman and colleagues.[37]

4.1.2 Hydantoins

A controversy regarding the fetal hydantoin syndrome has been raised. Hanson and Smith[78] reported the pattern of abnormalities in phenytoin-exposed neonates. The craniofacial features of these individuals were broad depressed nasal bridge, short upturned nose, hypertelorism, epicanthal folds and ptosis. Microcephaly, mental retardation and hypoplasia of the distal phalanges were also described. However, these features do not appear specific to phenytoin exposure since they have been described in the offspring of epileptic women treated with other AEDs.[27,82] Furthermore, Gaily et al.[83] recently reported that several anomalies previously regarded as typical fetal hydantoin syndrome (as described by Hanson and Smith[78]) were genetically linked to epilepsy. Only hypertelorism and digital hypoplasia were specifically associated with phenytoin exposure, suggesting that most of the typical characteristics were not caused by phenytoin. Further, Nakane et al.[38] found no significant association between phenytoin exposure and malformed infants.

Therefore, it is unlikely that the fetal hydantoin syndrome is a specific entity caused exclusively by phenytoin.

4.1.3 Valproic Acid (Sodium Valproate)

A pattern of major malformations specifically associated with valproic acid has been suggested. This includes abnormalities of the skeleton, CNS, cardiovascular and urogenital systems, and a variety of minor anomalies.[76] The main features of minor anomalies include brachycephaly, high forehead, shallow orbits, flat nasal bridge, small nose, hypertelorism, long upper lip, and low-set and rotated ears. However, the facial phenotype described by Di Liberti et al.[82] differs in many respects from features reported by Jäger-Roman and colleagues,[76] indicating that facial dysmorphism is frequent, but heterogeneous. Therefore, the fetal valproic acid syndrome is not yet substantiated as a specific set of anomalies caused by valproic acid exposure.

In summary, no firm AED–specificity, i.e. a direct association between a specific AED and a specific craniofacial malformation, has been established. However, substantial findings do support the fact that AEDs can be linked to several craniofacial malformations.

4.2 Neural Tube Defects

4.2.1 Valproic Acid

Valproic acid treatment during pregnancy may result in a 5- to 10-fold increase in the incidence of neural tube defect compared with the incidence in the general population.[84] The report from the International Clearing House pointed to a possible linkage between maternal valproic acid treatment and fetal neural tube defects.[85] The results summarised by Lindhout and Schmidt[86] indicated that valproic acid exposure during the first trimester of pregnancy was related to an increased risk of neural tube defects of 1.5% (95% confidence limits, 0.42 to 2.00%). Thus, the association between valproic acid exposure and neural tube effects was believed to be specific.

4.2.2 Carbamazepine

Rosa[87] has pointed out that carbamazepine exposure *in utero* without concurrent exposure to valproic acid carries a 1% risk of spina bifida, while exposure to valproic acid alone carries a 1.47% risk. This indicates that neural tube defects may not be exclusively specific to valproic acid exposure, and that carbamazepine exposure is also possibly associated with neural tube defects.

4.3 Teratogenicity of Newer AEDs

There are no available human data on the teratogenicity of the newer AEDs such as lamotrigine, vigabatrin and gabapentin. However, 20 epileptic mothers who were treated with zonisamide were investigated (2 who received the drug as monotherapy and 18 as part of a polypharmacy regimen), and only 1 case of malformation in the offspring was reported. This was atrial septal defect in the infant of a mother who was receiving daily doses of zonisamide 100mg, phenytoin 200mg and valproic acid 400mg (Kaneko et al. unpublished data). Thus, the specificity and frequency of malformations caused by the newer agents is, at present, unknown.

5. Risk Factors and Mechanisms of AED Teratogenesis

5.1 Dosage or Serum Drug Concentration

Nau et al.[13] showed the dose-dependent or peak concentration–dependent teratogenic effects of valproic acid in their animal experiments. Likewise, Dansky and coworkers[88] reported that the incidence of malformations in infants correlated positively with maternal phenytoin concentrations, and Jäger-Roman et al.[76] obtained similar results with valproic acid. Kaneko et al.[30] found an association between high total daily dosage of AEDs and the occurrence of malformations. Our recent international collaborative study[29] also showed that the dosage of valproic acid, as well as phenytoin, and serum valproic acid concentration were closely related to the occurrence of malformations in the offspring of patients receiving AED monotherapy (fig. 1).

These observations clearly indicate that high drug dosage or high serum drug concentration are risk factors for malformations, especially in those patients treated with valproic acid or phenytoin. In

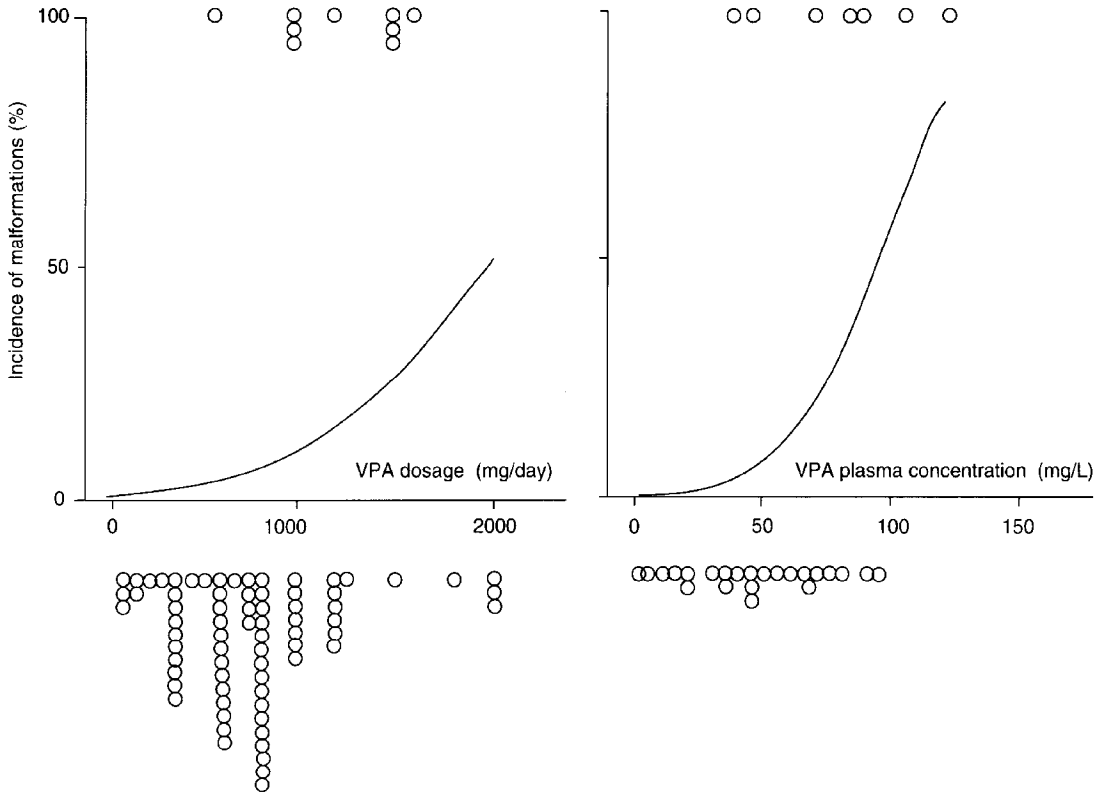

Fig. 1. Relationship between maternal valproic acid (sodium valproate) [VPA] dosage or plasma concentration and the occurrence of malformations in offspring of mothers treated with valproic acid as monotherapy. Ordinate lines show the cumulative malformation rates. The points above and below the lines indicate the presence or absence of malformations, respectively. Both dosage and drug concentration positively correlate with the occurrence of malformations.[29]

addition, the maximal concentration of valproic acid during the early stage of pregnancy correlated well with the incidence of valproic acid–induced neural tube defects, although the area under the serum concentration-time curve does not.[13,89] This result suggests the necessity of dividing the daily valproic acid dosage or replacing a conventional form of valproic acid with a controlled release form[90] to avoid high peak drug concentrations.

5.2 Drug Selection and Polypharmacy Patterns

When treating pregnant women who have epilepsy, the selection of AEDs or AED–combinations should be based on both a minimalisation of harmful effects on the developing fetus and effective seizure control for the mother.

A comparison of the teratogenic potency of major AEDs should be based on studies involving a sufficient number of patients receiving monotherapy (not polypharmacy). Our recent international collaborative study[29] fulfilled these conditions (by involving 495 patients who were treated with only 1 AED), and allowed us to rank the teratogenic potency of the major AEDs (table III). As can be seen, the order in incidences of malformations was primidone > valproic acid > phenytoin > carbamazepine > phenobarbital.

In the same study,[29] we observed a higher incidence of malformation in the polypharmacy group (10.6%) than in the monotherapy group (7.8%) and the number of AEDs was significantly

Table III. Incidences of malformations in the offspring of 495 patients receiving antiepileptic drug monotherapy and 97 untreated patients[29]

Drugs	Total number of mothers	No. of malformed offspring	Incidence (%)
Untreated			
Total	97	3	3.1
Treated			
Primidone	35	5	14.3
Valproic acid (sodium valproate)	81	9	11.1
Phenytoin	132	12	9.1
Carbamazepine	158	9	5.7
Phenobarbital (phenobarbitone)	79	4	5.1
Other antiepileptic drugs	10	0	0.0
Total	**495**	**39**	**7.9**

associated with the occurrence of malformations ($p < 0.035$), indicating that polypharmacy enhances the teratogenesis of AEDs. A high number of AEDs was also regarded as a drug-related risk factor for AED–induced teratogenesis.[29,31]

Among patients receiving AED polypharmacy, the incidences of malformations are remarkably high with some combinations. Lindhout et al.[91] reported a high frequency of congenital anomalies among infants exposed to valproic acid plus carbamazepine plus phenobarbital ± phenytoin.Similarly, Kaneko and colleagues[30] reported that valproic acid polypharmacy (valproic acid plus carbamazepine plus phenytoin ± other AEDs; valproic acid plus carbamazepine ± other AEDs; valproic acid plus phenytoin ± other AEDs) resulted in a high incidence of malformation. Our recent international collaborative study[29] also demonstrated a higher risk of congenital malformation with combinations such as valproic acid and carbamazepine, and phenytoin and/or carbamazepine ± barbiturates.

Therefore, AED polypharmacy is clearly related to an increased risk of teratogenicity, and particular attention should be paid to certain AED combinations that carry a very high risk. AED interactions during polypharmacy may possibly cause an increase in the concentrations of epoxides or other toxic metabolites (section 5.3.). In conclusion, if possible, AED polypharmacy should be changed to AED monotherapy before conception.

In addition, high doses or concentrations of AEDs and polypharmacy are often required in the treatment of refractory epilepsy. This suggests that these drug risk factors may partly reflect the teratogenic risk factors of epilepsy itself, although the risk of teratogenicity is mainly ascribable to AED factors rather than maternal risk factors.[19]

5.3 Epoxides and Other Toxic Metabolites

5.3.1 Phenytoin

Martz and coworkers[92] demonstrated that an oxidised metabolite of phenytoin binds to macromolecules (such as DNA, RNA and proteins) in mice fetuses, and that this binding is associated with an increased teratogenic action of phenytoin. These findings suggest that an intermediary metabolite, such as an epoxide, may be the causative teratogenic agent for some AEDs. Arene oxides (a type of epoxide), in addition to isomerising to phenols, react readily with a variety of nucleophiles, including cellular macromolecules such as DNA, RNA and proteins. Thus, arene oxide metabolites of aromatic compounds have become the prime candidates responsible for binding to biopolymers within the cell.

Phenytoin, carbamazepine and other AEDs are known to be potent inducers of liver microsomal oxidation in humans.[93,94] The human fetus has an hepatic and extrahepatic mono-oxygenase system,[95] which may catalyse the formation of epoxides,[96,97] and hepatic and extrahepatic enzymes that metabolise epoxides.[98,99] In the human liver, sulfhydryl groups and glutathione are also present, which react spontaneously or metabolically with reactive metabolites of drugs.[100] The balance between the enzyme activities catalysing the formation and the elimination of epoxides, therefore, could be a determinant of the probability of malformation.

Changes in epoxide hydrolase activity may be related to the teratogenic potential of some AEDs,

such as phenytoin and carbamazepine. For example, phenytoin itself enhances epoxide metabolism in cultured fetal hepatocytes,[101] and may also induce the metabolism of its own epoxide. It is interesting that the teratogenicity of AEDs has appeared in some, but not all, AED–exposed offspring. This suggests that a genetic defect of detoxification of arene oxide metabolites of phenytoin may increase the risk of the baby having major birth defects.[102] Recently, Buehler et al.[103] found that the mean epoxide hydrolase activity in amniocytes of mothers of offspring with the fetal phenytoin syndrome was significantly lower than that in normal pregnant women. This result suggests that this enzymatic biomarker may prove useful in determining which fetuses are at increased risk for congenital malformations caused by AEDs.

Furthermore, Finnell et al.[104] showed that coadministration of stiripentol (a potent inhibitor of cytochrome P450 enzymes) reduced fetal defects in mouse fetuses exposed to phenytoin, without affecting maternal plasma phenytoin concentrations. This suggests that oxidative metabolites activated by cytochrome P450 are the primary teratogenic molecules involved in phenytoin-induced teratogenesis.

5.3.2 Carbamazepine

Lindhout and colleagues[26,91] suspected that an accumulation of carbamazepine-10,11-epoxide or other epoxide intermediates caused by concomitant use of carbamazepine with phenobarbital, valproic acid and/or phenytoin could be teratogenic. In view of the known cytotoxic, teratogenic and mutagenic properties of some aromatic polycyclic hydrocarbons,[105,106] it is of potential concern that carbamazepine forms epoxide intermediates during its biotransformation. However, carbamazepine-10,11-epoxide may indeed be less toxic than carbamazepine at higher concentrations.[107] Nevertheless, a report by Rambec et al.[108] demonstrated that the addition of carbamazepine to valproic acid therapy resulted in unexpectedly high concentrations of carbamazepine-10,11-epoxide in the serum (up to 13 mg/L), which were associated with marked adverse effects in patients receiving this combination. Kerr et al.[109] observed that therapeutic concentrations of valproic acid inhibited the hydrolysis of carbamazepine-10,11-epoxide and styrene oxide in human liver microsomes and in preparations of purified human liver microsomal epoxide hydrolase. Following administration of carbamazepine-10,11-epoxide to volunteers, the clearance of the transdihydrodiol metabolite was decreased 20% by concomitant administration of valproic acid. Therefore, it might be speculated that oxcarbazepine, which does not produce the epoxide metabolite,[110] might have a lower risk of malformations. There is also a possibility of the formation of other unstable cytotoxic epoxide intermediates of carbamazepine.

5.3.3 Valproic Acid

The possible involvement of pharmacologically active metabolites of valproic acid in teratogenesis was reported by Nau and Loscher.[10] These investigators showed that the teratogenicity of the metabolite 2-propyl-4-pentanoic acid (4-en) was very similar to that of valproic acid. The incidence of malformations has been reported to be high in the infants of patients receiving valproic acid polypharmacy,[30] although concomitant use of other AEDs enhances valproic acid clearance and decreases serum concentrations of valproic acid. On the other hand, the metabolic conversion of valproic acid to the teratogenic metabolite, 4-en, is enhanced during valproic acid polypharmacy.[111] The metabolism of valproic acid to 4-en is also enhanced at high valproic acid concentrations,[111] which has been identified as an important risk factor for increased valproic acid teratogenicity in animal experiments[13,89] and human studies.[29]

These results seem to support the hypothesis that 4-en may contribute to valproic acid teratogenesis. However, actual concentrations of 4-en in human serum are much less than those of valproic acid (serum 4-en/valproic acid ratios ranged from 0.04 to 0.66% in patients with epilepsy).[111] Therefore, 4-en may not be a crucial factor for valproic acid teratogenicity, although the susceptibility of the human embryo to 4-en is unknown and

this factor cannot be excluded. In addition, the peak concentration and metabolic formation of 4-en, as well as the peak concentration of valproic acid, are significantly decreased by replacing conventional valproic acid formulations with a controlled release form.[90] Therefore, the use of controlled release forms of valproic acid might be useful in reducing valproic acid–induced teratogenesis.

5.4 Folate Deficiency and Impaired Folate Metabolism

Folate is a water soluble vitamin that is required for DNA synthesis and plays an important and basic role in cellular reproduction in mammals, including humans.[112] Meadow[33] was the first to speculate that AEDs might cause congenital malformations by inducing folate deficiency. Indeed, folate deficiency is seen in epileptic patients[113] and in pregnant epileptic women[114-116] who are receiving AEDs.

AEDs interfere with the conversion of folate to its active reduced form 5-methyltetrahydrofolic acid, which enters the cerebrospinal fluid.[117] Serum phenobarbital concentrations have an inverse correlation with serum folate concentrations in pregnant epileptic women,[118] and valproic acid also depresses folate concentrations in the blood of epileptic patients.[119] A significant association between malformations in offspring of epileptic mothers and reduced maternal folate concentrations due to AED therapy during pregnancy has also been reported.[114,116] Furthermore, periconceptual folate supplementation prevents some malformations, such as neural tube defects.[120,121]

Wegner and Nau[122] studied the mechanism of teratogenesis of valproic acid in a mouse model and found that coadministration of 5-formyltetrahydrofolate [calcium folinate (folinic acid)] reduced the incidence of neural tube defect. Furthermore, teratogenic doses of valproic acid reduced folate and 10-formyltetrahydrofolate levels and increased tetrahydrofolate levels, indicating the inhibition of glutamate formyl transferase by valproic acid. These investigators suggested that interference with embryonic folate metabolism might be an important aspect of valproic acid–induced teratogenesis.

Phenytoin inhibits the metabolism of folate.[16,113] Phenytoin and/or its arene oxide metabolite may directly decrease the levels of activated folates and *S*-adenosylmethionine, which are involved in the transfer of methyl groups, or decrease spermidine and spermine levels, derivatives of *S*-adenosylmethionine, which may be involved in the maintenance of activated folates.[123,124]

These data suggest that the effects of AEDs on the fetus might be related to their influence on folate metabolism.

6. Measures to Prevent AED Teratogenesis

Possible mechanisms of teratogenesis of AEDs are summarised in figure 2.[8,111,122,125] When treating pregnant women or women of childbearing age who have epilepsy, attention should be paid to these mechanisms and attempts made to avoid the risks of AED teratogenesis. AED–associated factors are considered to be the primary risk factors for teratogenesis, although other risk factors in the multiple aetiology of congenital malformations must also be considered.

The prevention of birth defects can be attempted before conception by reducing the dosages of AEDs to the lowest dosages that effectively control seizures. A decrease in serum AED concentrations during pregnancy does not in itself justify an increase in dosage. Based on an analysis of 968 live born AED–exposed offspring,[126] there seems to be a cut-off value for daily dosage of certain AEDs regarding the occurrence of congenital malformations. From the study of offspring exposed to valproic acid monotherapy, 89% of malformations occurred at a dosage of 1000 mg/day or more.[126] Likewise, these percentages were 88% for carbamazepine ≥ 400 mg/day, 100% for primidone ≥ 400 mg/day and 90% for phenytoin ≥ 200 mg/day.[126]

Where possible, AED polypharmacy should be changed to monotherapy. In patients treated with both combinations of valproic acid plus carbamazepine, and phenytoin plus barbiturates, the high

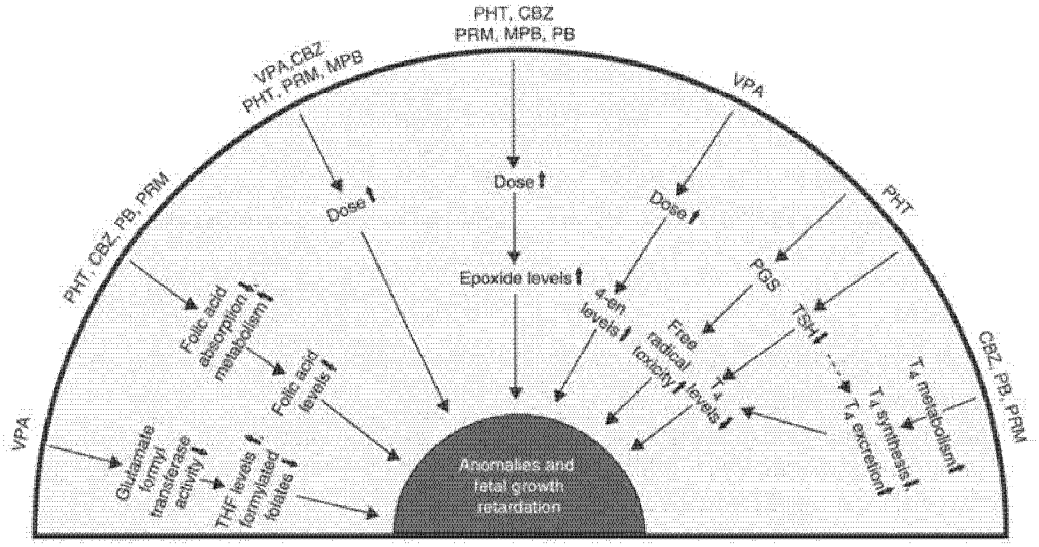

Fig. 2. Schematic presentation of possible mechanisms of teratogenesis of antiepileptic drugs (AEDs). PHT, CBZ, PB and PRM decrease folate levels, while VPA interferes with folate metabolism by inhibition of glutamate formyl transferase.[121] VPA, CBZ, PHT, PRM and MPB increase malformations in a dose-dependent manner.[8] Increased doses of PHT, CBZ, PRM, MPB and PB result in an increased formation of toxic metabolites such as epoxide intermediates,[8] while increasing the dosage of VPA results in an increased formation of the teratogenic metabolite 2-propyl-4-pentanoic acid (4-en).[111] Formation of 4-en is also enhanced by coadministration of VPA with other AEDs.[111] Conversely, coadministration of VPA inhibits detoxification of the epoxide intermediates of other AEDs. PGS–dependent free radical–mediated toxicity may be related to PHT teratogenesis.[125] All these factors may contribute to AED teratogenesis. Intrauterine growth retardation (in particular, small fetal head size) can be ascribed partly to a CBZ-, PHT-, PB- and PRM-induced reduction of thyroxin (T_4) levels.[8]
Abbreviations and symbols: CBZ = carbamazepine; MPB = methylphenobarbital (mephobarbital); PB = phenobarbital (phenobarbitone); PGS = prostaglandin synthetase; PHT = phenytoin; PRM = primidone; THF = tetrahydrofolate; TSH = thyroid-stimulating hormone; VPA = valproic acid (sodium valproate); small ↑ indicates an increase; small ↓ indicates a decrease.

risk of malformation should be taken into account. Special attention should be paid to the detection of malformation if these combinations of AEDs are unavoidable. If very severe malformations are observed, the necessary measures should be taken.

A 1 to 2% risk of neural tube defects occurs in offspring of mothers treated with valproic acid (1 to 2%) or carbamazepine (0.9%). As stated by the International League Against Epilepsy,[127] this risk justifies offering prenatal diagnosis with ultrasound and/or amniotic fluid analysis of α-fetoprotein. This approach can also be taken in patients with a family history of neural tube defects. The risk of malformations such as heart defects and facial clefts also warrants offering prenatal diagnosis by ultrasound.

If possible, replacement of valproic acid with another AED is advisable. However, if this is not possible, valproic acid monotherapy must be considered. A single daily dose regimen of valproic acid is not advisable[13,89] because the adverse effects result from unpredictably high peak plasma drug concentrations. The use of the controlled release form of valproic acid may be helpful in decreasing valproic acid teratogenicity, since the peak concentration of valproic acid and its toxic metabolite is greatly reduced compared with conventional preparations of valproic acid.[90]

As mentioned in section 5.4, it is possible that AED–associated malformations, including neural tube defects, might be related to folate deficiency or impaired folate metabolism. Therefore, the routine provision of folate supplements, preferably

with an already reduced form of folate such as 5-formyltetrahydrofolate, to women on AED medication before conception is recommended. This assumes that the animal findings of Wegner and Nau[122] and of Olney's group,[128] in which the reduced metabolite 5-formyltetrahydrofolate was less neurotoxic than folate itself, are also true for humans.

7. Conclusions

Offspring of AED–treated epileptic mothers have a higher risk of malformation than those of untreated epileptic mothers and of the general population. AEDs are major risk factors for this. Thus, this review has focused on the teratogenicity of AEDs, and has discussed this issue from various aspects. Although the mechanisms of AED–induced teratogenesis are still controversial and speculative, several AED risk factors have been extracted from a large number of clinical investigations. Based on these accumulated findings, we should be more careful about the choice, dosage and combination patterns of AEDs in the treatment of epileptic women of childbearing age, and should take necessary measures to prevent AED teratogenesis. It is also expected that future studies will lead to better solutions for the clinically important issue of how to reduce the risk of birth defects in pregnant epileptic women who require AED therapy.

Acknowledgements

This study was supported by a Grant from the Pharmacopsychiatry Research Foundation, and a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture (05454309). We are also grateful to Dr Tony S. Rausch for his useful discussion.

References

1. Ogawa Y, Nomura Y, Kaneko S, et al. Insidious effect of antiepileptic drugs in the perinatal period. In: Janz D, Dam M, Richens A, et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 197-202
2. Kaneko S, Otani K, Fukushima Y, et al. Transplacental passage and half-life of sodium valproate in infants born to epileptic mothers. Br J Clin Pharmacol 1983; 15: 503-5
3. Nau H, Rating D, Koch S, et al. Valproic acid and its metabolites: placental transfer, neonatal pharmacokinetics, transfer via mother's milk and clinical status in neonates of epileptic mothers. J Pharmacol Exp Ther 1981; 291: 768-77
4. Kojima S, Ogura H, Yokoyama M, et al. The effect of diazepam on neonatal neurobehavioral response. Acta Obstet Gynecol Jpn 1985; 37: 2784-90
5. Nau H, Helge H, Luck W. Valproic acid in the perinatal period: decreased maternal serum protein binding results in fetal accumulation and neonatal displacement of the drug and some metabolites. J Pediatr 1984; 104: 627-34
6. Nau H, Krauer B. Serum protein binding of valproic acid in fetus-mother pairs throughout pregnancy: correlation with oxytocin administration and albumin and free fatty acid concentrations. J Clin Pharmacol 1986; 26: 215-21
7. Cotariu D, Zaidman JL. Developmental toxicity of valproic acid. Life Sci 1991; 48: 1341-50
8. Kaneko S. Antiepileptic drug therapy and reproductive consequence: functional and morphologic effects. Reprod Toxicol 1991; 5: 179-98
9. Sullivan FM, McElhatton PR. Teratogenic activity of the antiepileptic drugs: phenobarbital, phenytoin, and primidone in mice. Toxicol Appl Pharmacol 1975; 34: 271-82
10. Nau H, Loscher W. Valproic acid and metabolites: pharmacological and toxicological studies. Epilepsia 1984; 25: S14-22
11. Massey KM. Teratogenic effects of diphenylhydantoin sodium. Oral Ther Pharmacol 1966; 2: 380-5
12. Brown NA, Shull G, Fabro S. Assessment of the teratogenic potential of trimethadione in the CD-1 mouse. Toxicol Appl Pharmacol 1979; 51: 59-71
13. Nau H, Zierer R, Spielmann D, et al. A new model for embryotoxicity testing: teratogenicity and pharmacokinetics of valproic acid following constant-rate administration in the mouse using human therapeutic drug and metabolite concentrations. Life Sci 1981; 29: 2803-14
14. Bergman A, Rosseli-Austin L, Yedwab G, et al. Neuronal deficits in mice following phenobarbital exposure during various periods in fetal development. Acta Anat 1980; 108: 370-3
15. Shah RM, Donaldson D, Burdett D. Teratogenic effects of diazepam in the hamster. Can J Physiol Pharmacol 1979; 57: 556-61
16. Chatot CL, Klein NW, Clapper ML, et al. Human serum teratogenicity studied by rat embryo culture: epilepsy, anticonvulsant drugs, and nutrition. Epilepsia 1984; 25: 205-16
17. South J. Teratogenic effect of anticonvulsants [letter]. Lancet 1972; 2: 1154
18. Kuenssberg EV, Knox JDE. Teratogenic effect of anticonvulsants [letter]. Lancet 1973; 1: 198
19. Hill RM, Verniaud VM, Rettig GM, et al. Relationship between antiepileptic drug exposure of the infant and development potential. In: Janz D, Dam M, Richens A, et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 409-17
20. Goujard J, Huel G, Rumeau-Roquette C. Antiepileptiques et malformations congenitales. J Gynecol Obstet Biol Reprod 1974; 3: 831-42
21. Shapiro S, Hartz SC, Siskind V, et al. Anticonvulsants and parental epilepsy in the development of birth defects. Lancet 1976; 1: 272-5
22. Granstrom ML, Hiilesmaa VK. Malformations and minor anomalies in the children of epileptic mothers: preliminary results of the prospective Helsinki study. In: Janz D, Dam D, Richens A, et al. editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 303-7
23. Nakane Y. Factors influencing the risk of malformation among infants of epileptic mothers. In: Janz D, Dam D, Richens A,

et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 259-65
24. Koch S, Hartmann AM, Jager-Roman E, et al. Major malformations in children of epileptic mothers – due to epilepsy or its therapy?. In: Janz D, Dam D, Richens A. et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 313-5
25. Bossi L, Battino D, Boldi B, et al. Anthropometric data and minor malformations in newborns of epileptic mothers. In: Janz D, Dam D, Richens A, et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 299-301
26. Lindhout H, Meinardi H, Barth PG. Hazards of fetal exposure to drug combinations. In: Janz D, Dam D, Richens A, et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 275-81
27. Miyakoshi M, Seino M. Malformations in children born to mothers with epilepsy. In: Sato T, Shinagawa S, editors. Antiepileptic drugs and pregnancy. Amsterdam: Excerpta Medica, 1984: 125-31
28. Kaneko S, Fukushima Y, Sato T, et al. Teratogenicity of antiepileptic drugs – a prospective study. Jpn J Psychiatry Neurol 1986; 40: 447-50
29. Kaneko S, Battino D, Andermann E, et al. International collaborative study on mechanisms of teratogenesis of antiepileptic drugs (a preliminary report) [in Japanese, abstract in English]. Ann Rep Jpn Epil Res Found 1993; 5: 191-9
30. Kaneko S, Otani K, Fukushima Y, et al. Teratogenicity of antiepileptic drugs: analysis of possible risk factors. Epilepsia 1988; 29: 459-67
31. Kaneko S, Otani K, Kondo T, et al. Malformation in infants of mothers with epilepsy receiving antiepileptic drugs. Neurology 1992; 42 Suppl. 5: 68-74
32. Bossi L. Fetal effects of anticonvulsants. In: Morselli PL, Pippenger CE, Penry JK, editors. Antiepileptic drug therapy in pediatrics. New York: Raven Press, 1983: 37-64
33. Meadow SR. Anticonvulsant drugs and congenital abnormalities [letter]. Lancet 1968; 2: 1296
34. Beck-Mannagetta G, Drees B, Janz D. Malformations and minor anomalies in the offspring of epileptic patients. In: Janz D, Dam M, Richens A, et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 317-23
35. German J, Ehlers KM, Kowal A, et al. Possible teratogenicity of the trimethadione and paramethadione. Lancet 1970; 2: 261-2
36. Zachai EH, Mellman WJ, Niederren B, et al. The fetal trimethadione syndrome. J Pediatr 1975; 87: 280-4
37. Feldman GL, Weaver DD, Lovrien EW. The fetal trimethadione syndrome. Report of additional family and further delineation of this syndrome. Am J Dis Child 1977; 131: 1389-92
38. Nakane Y, Okuma T, Takahashi R, et al. Multi-institutional study on the teratogenicity and fetal toxicity of antiepileptic drugs: a report of a collaborative study group in Japan. Epilepsia 1980; 21: 663-80
39. Andermann E, Dansky L, Andermann F, et al. Minor congenital malformations and dermatoglyphic alterations in the offspring of epileptic women. In: Janz D, Dam M, Richens A,et al., editors. Epilepsy, pregnancy and the child. New York: Raven Press, 1982: 235-49
40. Anderson RC. Cardiac defects in children of mothers receiving anticonvulsant therapy during pregnancy. J Pediatr 1976: 89 318-9
41. Annegers JF, Elvebeck LR, Hauser WA, et al. Do anticonvulsants have a teratogenic effect? Arch Neurol 1974; 31: 364-73
42. Annegers JF, Elvebeck LR, Hauser WA, et al. Congenital malformations and seizure disorders in the offspring of parents with epilepsy. Int J Epidemiol 1978; 7: 241-7
43. Barr M, Posnanski AK, Schmickel RD. Digital hypoplasia and anticonvulsants during gestation: a teratogenic syndrome? J Pediatr 1974; 84: 254-6
44. Barry JE, Danks DM. Anticonvulsants and congenital abnormalities. Lancet 1974; 2: 48-9
45. Baughman FA, Randinitis EJ. Passage of DPH across the placenta [letter]. JAMA 1970; 213: 466
46. Bethenod M, Fredreich A. Les enfants des antiepileptiques. Pediatrie 1975; 30: 227-48
47. Biale Y, Lewenthal H, Aderet NB. Congenital malformations due to anticonvulsive drugs. Obstet Gynecol 1975; 45: 439-42
48. Bjerkedal T. Outcome of pregnancy in women with epilepsy. Norway 1968-1978: congenital malformations. In: Janz D, Bossi L, Dam M, et al., editors. Epilepsy, pregnancy and the child. New York: Raven Press, 1982; 289-95
49. Dansky L. Andermann E, Sherwin AL, et al. Maternal epilepsy and birth defects: a prospective study with monitoring of plasma anticonvulsant levels during pregnancy. In: Dam M, Gram L, Penry JK, et al., editors. Advances in epileptology, XIIth Epilepsy International Symposium. New York: Raven Press, 1981; 607-12
50. Desmond HM. Maternal barbiturate utilization and neonate withdrawal symptomatology. J Pediatr 1972; 80: 190-7
51. Elshove J, Van Eck JHM. Aageboren misvormingen, mentam gespleten lip met of zonder gespleten verhemelte, bij kindarer van moeders met epilepsie. Ned Tijdschr Geneeskd 1971; 115: 1371-5
52. Fedrick J. Epilepsy and pregnancy. A report from the Oxford record linkage study. BMJ 1973; 2: 442-8
53. Hanson JW, Myrianthopoulos NC, Sedgwick Harvey MA, et al. Risks to the offspring of women treated with hydantoin anticonvulsants, with emphasis on the fetal hydantoin syndrome. J Pediatr 1976; 89: 662-8
54. Hill RM, Verniaud WM, Horning MD, et al. Infants exposed *in utero* to antiepileptic drugs. Am J Dis Child 1974; 127: 645-53
55. Janz D, Fuchs U. Sind antiepileptitishe Medikamente während der Schwangerschaft schädlich? Dtsch Med Wochenschr 1964; 89: 241-3
56. Koppe JG, Bosman W, Oppers VM, et al. Epilepsie en aangeboren afwijkingen. Ned Tijdschr Geneeskd 1973; 117: 220-4
57. Loughnan PM, Gold H, Vance JC. Phenytoin teratogenicity in man. Lancet 1973; 1: 70-2
58. Lowe CR. Congenital malformations among infants born to epileptic women. Lancet 1973; 1: 9-10
59. Maroni E, Markoff R. Epilepsie und Schwangerschaft. Gynaecologia 1969; 168: 418-21
60. McMullin GP. Teratogenic effects of anticonvulsants. BMJ 1971; 4: 430
61. Meadow SR. Congenital abnormalities and anticonvulsant drugs. Proc R Soc Med 1970; 63: 48-9
62. Melchior IC, Svensmark O, Trolle D. Placental transfer of phenobarbitone in epileptic women, and elimination in newborns. Lancet 1967; 2: 860-1
63. Meyer JG. The teratological effects of anticonvulsants and the effects on pregnancy and birth. Eur Neurol 1973; 10: 179-90
64. Millarr HG, Nevin NC. Congenital malformations and anticonvulsant drugs [letter]. Lancet 1973; 1: 328

65. Mirkin BL. Diphenylhydantoin: placental transport, fetal localization, neonatal metabolism, and possible teratogenic effect. J Pediatr 1971; 78: 329-37
66. Monson RR, Rosenberg L, Hartz SC, et al. Diphenylhydantoin and selected congenital malformations. N Engl J Med 1973; 289: 1049-52
67. Pashayan H, Pruzansky D, Pruzansky S. Are anticonvulsants teratogenic? Lancet 1971; 2: 702-3
68. Speidel BD, Meadow SR. Maternal epilepsy and abnormalities of the fetus and the newborn. Lancet 1972; 2: 839-43
69. Starreveld-Zimmermann AAE, Van der Kolk WJ, Meinardi H, et al. Are anticonvulsants teratogenic? [letter] Lancet 1973; 2: 48
70. Villumsen Al, Zacau-Christiansen B. Incidence of malformations in the newborn in a prospective child health study. Bull Soc R Belge Gynecol Obstet 1973; 23: 95
71. Watson ID, Spellacy WN. Neonatal effects of maternal treatment with the anticonvulsant drug diphenylhydantoin. Obstet Gynecol 1971; 37: 881-5
72. Weber M, Schweitzer M, Mur JM, et al. Epilepsie, medicaments antiepileptiques et grossesse. Arch Fr Pediatr 1977; 34: 374-83
73. Janz D. On major malformations and minor anomalies in the offspring of parents with epilepsy. In: Janz D, Dam M, Richens A, et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 221-2
74. Friis ML. Epilepsy among parents of children with facial clefts. Epilepsia 1979; 20: 69-76
75. Friis ML, Holm NV, Sindrup EH, et al. Facial clefts in siblings and children of epileptic patients. Neurology 1986; 36: 346-50
76. Jäger-Roman E, Deichi A, Jacob S, et al. Fetal growth, major malformations, and minor anomalies in infants born to women receiving valproic acid. J Pediatr 1986; 108: 997-1004
77. Rating D, Jager-Roman E, Koch S, et al. Minor anomalies in the offspring of epileptic parents. In: Janz D, Dam M, Richens A, et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 283-8
78. Hanson JW, Smith DW. The fetal hydantoin syndrome. J Pediatr 1975; 87: 285-90
79. Seip M. Growth retardation, dysmorphic facies and minor malformations following massive exposure to phenobarbitone *in utero*. Acta Paediatr Scand 1976; 65: 617-21
80. Smith DW. Teratogenicity of anticonvulsive medications. Am J Dis Child 1977; 131: 1337-9
81. Goldman AS, Yaffe SJ. Fetal trimethadione syndrome. Teratology 1978; 17: 103-6
82. Di Liberti JM, Farndon PA, Dennis NR. The fetal valproate syndrome. Am J Med Genet 1984; 19: 473-81
83. Gaily E, Granstrom M-L, Hiilesmaa V, et al. Minor anomalies in offspring of epileptic mothers. J Pediatr 1988; 112: 520-9
84. Robert E, Rosa F. Valproate and birth defects [letter]. Lancet 1983; 2: 1142
85. Robert E, Guiboud P. Maternal valproic acid and congenital neural tube defects [letter]. Lancet 1982; 2: 937
86. Lindhout D, Schmidt D. *In-utero* exposure to valproate and neural tube defects [letter]. Lancet 1986; 1: 1392-3
87. Rosa FW. Spina bifida in infants of women treated with carbamazepine during pregnancy. N Engl J Med 1991; 342: 674-7
88. Dansky L, Andermann E, Andermann F. Maternal epilepsy and congenital malformations: correlation with maternal plasma anticonvulsant levels during pregnancy. In: Janz D, Dam M, Richens A, et al., editors. Epilepsy, pregnancy, and the child. New York: Raven Press, 1982: 251-8
89. Nau H. Teratogenic valproic acid concentrations: infusion by implanted mini pumps vs conventional injection regimen in the mouse. Toxicol Appl Pharmacol 1985: 80; 243-50
90. Kondo T, Kaneko S. Teratogenicity of valproate pharmacokinetic aspects [abstract]. Neuropsychopharmacology 1994; 10 Suppl. 3: 573S
91. Lindhout D, Hoppener RJEA, Meinardi H. Teratogenicity of anti-epileptic drug combinations with special emphasis on epoxidation (of carbamazepine). Epilepsia 1984; 25: 77-83
92. Martz F, Failinger III C, Blake DA. Phenytoin teratogenesis: correlation between embryopathic effect and covalent binding of putative arene oxide metabolite in gestational tissue. Pharmacol Exp Ther 1977; 203: 231-9
93. Conney AH. Microsomal enzyme induction by drugs. Pharmacol Physicians 1969; 3: 1-6
94. Remmer H, Fleischmann R, Kunz W. Pharmacological consequences of induction of drug metabolizing enzymes. In: Estabrook RW, Lindenlaub E, editors. The induction of drug metabolism. Stuttgart: Schattauer, 1979: 555-81
95. Yaffe SJ, Rane A, Sjoqvist F, et al. The presence of a monooxygenase system in human fetal liver microsomes. Life Sci 1970; 9: 1189-200
96. Pelkonen D, Karki NT. Epoxidation of xenobiotics in the human fetus and placenta: a possible mechanism of transplacental drug-induced injuries. Biochem Pharmacol 1975; 24: 1445-8
97. Piafsky KM, Rane A. Formation of carbamazepine epoxide in human fetal liver. Drug Metab Dispos 1978; 6: 502-3
98. Pacifici GM, Norlin A, Rane A. Glutathione S-transferase in human fetal liver. Biochem Pharmacol 1981; 30: 3367-71
99. Pacifici GM, Rane A. Epoxide hydrolase in human fetal liver. Pharmacology 1983; 26: 241-8
100. Rollins D, Larsson A, Steen B, et al. Glutathione and gamma-glutamyl cycle enzymes in fetal liver. J Pharmacol Exp Ther 1981; 217: 697-700
101. Rane A, Peng D. Phenytoin enhances epoxide metabolism in human, fetal liver culture. Drug Metab Dispos 1985; 13: 382-5
102. Strickler SM, Miller MA, Andermann E, et al. Genetic predisposition to phenytoin-induced birth defects. Lancet 1985; 2: 746-9
103. Buehler BA, Delimont D, Van Waes M, et al. Prenatal prediction of risk of the fetal hydantoin syndrome. N Engl J Med 1990; 322: 1567-72
104. Finnell RH, Kerr BM, Van Waes M, et al. Protection from phenytoin-induced congenital malformations by coadministration of the antiepileptic drug stiripentol in a mouse model. Epilepsia 1994; 35: 141-8
105. Jerina DM, Daly JW. Arene oxides: a new aspect of drug metabolism; metabolic formation of arene oxide explains many toxic and carcinogenic properties of aromatic hydrocarbons. Science 1974; 185: 573-82
106. Spielberg SP, Gordon GB, Blake DA, et al. Anticonvulsant toxicity in vitro: possible role of arene oxides. J Pharmacol Exp Ther 1981; 217: 386-9
107. Frigerio A, Morselli PL. Carbamazepine: biotransformation. Adv Neurol 1975; 11: 295-308
108. Rambec B, Salke-Treumann A, May T, et al. Valproic acid-induced carbamazepine-10,11-epoxide toxicity in children and adolescents. Eur Neurol 1980; 30: 79-83
109. Kerr BM, Rettie AE, Eddy AC, et al. Inhibition of human liver microsomal epoxide hydrolase by valproate and valpromide: *in vitro/in vivo* correlation. Clin Pharmacol Ther 1989; 46: 82-93

110. Theisohn M, Meimann G. Disposition of the antiepileptic oxcarbazepine and its metabolites in healthy volunteers. Eur J Clin Pharmacol 1982; 22: 545-51
111. Kondo T, Kaneko S, Otani K, et al. Associations between risk factors for valproate hepatotoxicity and altered valproate metabolism. Epilepsia 1992; 33: 172-7
112. Kitay DZ. Folic acid and reproduction. Clin Obstet Gynecol 1979; 22: 809-17
113. Reynolds EH. Anticonvulsants, folic acid, and epilepsy. Lancet 1973; 1: 1376-8
114. Dansky LV, Andermann E, Rosenblatt D, et al. Anticonvulsants, folate levels, and pregnancy outcome: a prospective study. Ann Neurol 1987; 21: 176-82
115. Yamamoto M, Takahashi S, Otsuki S, et al. Serum folate levels during pregnancy and after delivery in treated epileptic women: a preliminary report. In: Sato T, Shinagawa S, editors. Antiepileptic drugs and pregnancy. Amsterdam: Excerpta Medica, 1984: 163-7
116. Kaneko S, Otani K, Hirano T, et al. Mechanism of teratogenicity of antiepileptic drugs. Third report: serum folic acid levels during pregnancy. Ann Rep Pharmacopsychiat Res Found 1988; 19: 306-11
117. Woodbury DM, Kemp JM. Pharmacology and mechanisms of action of diphenylhydantoin. Psychiatr Neurol Neurochem 1971; 74: 91-115
118. Hiilesmaa VK, Teramo K, Granstrom M-L, et al. Serum folate concentrations during pregnancy in women with epilepsy: relation to antiepileptic drug concentrations, number of seizures, and fetal outcome. BMJ 1983; 287: 577-9
119. Berg MJ, Fincham RW, Ebert BE, et al. Decrease of serum folates in healthy male volunteers taking phenytoin. Epilepsia 1988; 29: 67-73
120. Laurence KM, James N, Miller MH, et al. Double-blind randomized controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. BMJ 1981; 282: 1509-11
121. Smithells RW, Sheppard S, Schorah CJ, et al. Apparent prevention of neural tube defects by periconceptional vitamin supplementation. Arch Dis Child 1981; 56: 911-8
122. Wegner C, Nau H. Alteration of embryonic folate metabolism be valproic acid during organogenesis: implications for mechanisms of teratogenesis. Neurology 1992; 42 Suppl. 5: 17-24
123. Netzloff ML, Streiff RR, Frias JL, et al. Folate antagonism following teratogenic exposure to diphenylhydantoin. Teratology 1979; 19: 45-50
124. Wells PG, Harbison RD. Significance of the phenytoin reactive arene oxide intermediate, its oxepin tautomer, and clinical factors modifying their roles in phenytoin-induced teratology. In: Hassell TH, Johnston MC, Dudley KH, editors. Phenytoin-induced teratology and gingival pathology. New York: Raven Press, 1980: 83-112
125. Wells PG, Zubovits JT, Wong ST, et al. Modulation of phenytoin teratogenicity and embryonic covalent binding by acetylsalicylic acid and alpha-phenyl-N-t-butylnitrone: implications for bioactivation by prostaglandin synthetase. Toxicol Pharmacol 1989; 97: 192-202
126. Kaneko S. Major malformations in children of mothers with epilepsy [abstract]. Neuropsychopharmacology 1994; 10 Suppl. 3: 576S
127. Commission on Genetics, Pregnancy, and the Child, International League Against Epilepsy. Guidelines for the care of women of childbearing age with epilepsy. Epilepsia 1993; 34: 588-9
128. Olney JW, Fuller TA, de Gubareff T. Kainate-like neurotoxicity of folate. Nature 1981; 292: 165-7

Correspondence and reprints: Dr *Sunao Kaneko*, Department of Neuropsychiatry, Hirosaki University, Hirosaki 036, Japan.